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USE OF FERROUS SULPHATE TO REDUCE THE REDOX POTENTIAL AND ALLOW THE START-UP OF UASB REACTORS TREATING SLOWLY BIODEGRADABLE COMPOUNDS: application to a wastewater containing 4-methylbenzoic acid

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ABSTRACT

During the anaerobic treatment of slowly biodegradable wastewaters containing dissolved oxygen, the natural communities of aerobic and anaerobic facultative bacteria present in reactors' sludges may not succeed in lowering the redox potential of their environment to a level compatible with methanogenesis. In order to allow reactor start-up, it is proposed to add a single initial dose of $FeSO_4$ directly to the reactor liquor, together with an inexpensive easily biodegradable compound (i. e. carbohydrates, agroindustrial wastes...). Under the action of sulphate reducing bacteria, $FeSO_4$ is reduced to FeS which in turn lowers redox potential. This method was tested on an Upflow Anaerobic Sludge Bed (UASB) reactor fed at an hydraulic retention time of 5.3 ± 0.3 days with an aerobic synthetic wastewater (dissolved O_2, 5 $mg.l^{-1}$; redox potential referred to the standard hydrogen electrode (E_h), +119 to +250 mV; pH 7 to 7.4; 6°C) containing *p*-toluic acid (4-methylbenzoic acid) as sole carbon and energy source. During the first 37 days of reactor operation, sludge E_h was high (+118 to +161 mV; pH 7.2 to 7.4; 30 to 35°C) and no COD (Chemical Oxygen Demand) removal occured. Soon after the addition of $FeSO_4$ (0.15 g $FeSO_4$. $7H_2O$ per g of Volatile Suspended Solids [VSS]) and glucose (0.38 g per g VSS), sludge E_h decreased to -95 mV (pH 7.3; 33°C) while COD removal increased to 51.3%. An economical way to implement this method at full scale is suggested.

KEYWORDS

Anaerobic wastewater treatment, UASB reactor, redox potential, start-up, ferrous sulphate, *p*-toluic acid

INTRODUCTION

Since the early work of Hungate and collaborators, it is well known that methanogenic bacteria are strict anaerobes, extremely sensitive to oxygen (1, 2). It is also generally considered that they cannot initiate growth if the E_h (oxidation reduction potential referred to the standard hydrogen electrode) of the surrounding environment is greater than -330 mV (1, 3).

Due to contact with air, most wastewaters contain dissolved oxygen and have positive E_h . This does not affect the anaerobic treatment of readily biodegradable effluents (i. e. agroindustrial wastewaters, sewage...) because dissolved oxygen is consumed by the aerobic and facultative anaerobic bacteria present in reactor sludges (4, 5), leading to a rapid fall in redox potential. In contrast, when wastewaters contain slowly biodegradable compounds (i. e. aromatic molecules), the electron flow generated during their degradation may not be sufficient to obtain an efficient oxygen reduction by the aerobes. In that case, redox

potential within digester sludges remains too high for the initiation of methanogenic fermentation preventing satisfactory reactor start-up.

When such a problem occurs, the only strategy possible consists of lowering the digester redox potential. This can be done in two ways: (i) via the addition to the wastewater of an easily metabolizable substrate like glucose in order to favour microbial oxygen consumption, or (ii) via the addition of a reducing agent in order to consume the oxygen chemically. Adequate reducing agents are sodium thioglycolate, cysteine, dithionite, sodium sulphide (3) and titanium (III) citrate (6). These agents have to be used with care since they are toxic at relatively low concentrations (3, 7) or produce very toxic oxidized derivatives when exposed to oxygen (8).

Ferrous sulphate, which is not toxic except at high concentrations (9), could replace advantageously the previous chemicals. It has no reducing power by itself; however, when fed to an anaerobic reactor, it is reduced to ferrous sulphide by the sulphate-reducing bacteria always present in reactors' inocula (10, 11). The resulting FeS is an excellent reducing agent which reacts with O_2 much more rapidly than either sodium sulphide or cysteine (12). Contrarily to Na_2S, it is also not toxic to methanogenic bacteria (13, 14). Finally, FeS is characterized by a very low solubility product (Ksp = 3.7 10^{-19}, 18°C) and a high specific gravity (4.74) leading to the formation of dense precipitates (15). For Upflow Anaerobic Sludge Bed (UASB) reactors, where the biomass is mainly located at the bottom of the digester, the FeS produced should then accumulate in the sludge bed and strongly buffer it at an adequate redox potential. Such accumulation could present a second advantage since FeS is suspected to increase the stability of sludge granules (16).

This study was part of a global research programme set up to understand the reasons behind the low performances of UASB reactors treating the wastewater generated by the terephthalic acid production industry (17). It consisted of operating a UASB reactor with a synthetic wastewater containing *p*-toluic acid (4-methylbenzoic acid, one of the main pollutants found in the previous waste) as sole carbon and energy source. Despite the known anaerobic biodegradability of this compound (18, 19), the reactor did not start-up since the redox potential of the sludge remained positive. The possibility of using limited amounts of ferrous sulphate and glucose to lower redox potential and thus allow reactor start-up was investigated.

MATERIALS AND METHODS

Laboratory scale UASB reactor set-up;

The experimental apparatus consisted of a 3.6 l glass column reactor (internal diameter: 9.6 cm, height 45 cm, conic base) fed continuously by way of a 1-100 rpm Masterflex® peristaltic pump. The reactor, operated without recirculation, was located in a controlled temperature room at 35°C. Its top was equipped with a gas-liquid-solid separator. The biogas produced was collected and measured as previously described (17).

Synthetic wastewater composition and preparation;

The defined medium was prepared with tap water and kept at 6°C under agitation. In such conditions, it contained around 5 mg l^{-1} of dissolved oxygen and presented a mean E_h of 186 ± 51 mV (pH 7.1 ± 0.3). Except for the concentrations of some components (*p*-toluic acid, $NaHCO_3$, glucose, $FeSO_4.7H_2O$) which were modified during the reactor operation (table 1), the synthetic medium contained in mg l^{-1}: NH_4Cl (660), $(NH_4)_2SO_4$ (250), K_2HPO_4 (130), KH_2PO4 (100), $CaCl_2$ (200), $MgCl_2.6H_2O$ (100), $(NH_4)_6Mo_7O_{24}.4H_2O$ (10), $ZnCl_2$ (1), $CoCl_2.6H_2O$ (1), $MnCl_2.4H_2O$ (1), total iron (0.4, coming from the tap water), $NiCl_2.6H_2O$ (0.2), $AlK(SO_4)_2.12H_2O$ (0.1) and $CuCl_2.2H_2O$ (0.05). Since it is difficult to solubilize *p*-toluic acid at room temperature, it was dissolved in boiling water prior to the addition of the other components (19).

Reactor start-up and operation.

The reactor was inoculated with 6.8 g VSS (Volatile Suspended Solids) of an anaerobic granular sludge from a laboratory UASB digester treating the wastewater of a terephthalic acid production factory.

Immediately after seeding, the reactor was fed at a Hydraulic Retention Time (HRT) of 5.3 ± 0.3 days which was maintained for the rest of the experiment.

Table 1. Summary of changes in wastewater composition

Compounds	Feeding periods					
	1 (day 0-6)	2 (day 7-11)	3 (day 12-19)	4 (day 20-34)	5 (day 35-97)	6 (day 98-269)
p-toluic acid ($g.l^{-1}$)	1	1	1	0.5	0.5	0.5
$NaHCO_3$ ($g.l^{-1}$)	1	1	1	0.5	0.5	0.5
glucose ($g.l^{-1}$)	0	0.1	0.1	0.1	0	0
$FeSO_4.7H_2O$ ($g.l^{-1}$)	0	0	0.1	0.1	0.1	0

Measurement of oxidation-reduction potential (ORP).

ORP was determined with an epoxy-body combination electrode (Cole-Parmer N-05990-55, Chicago, Illinois, USA) made of a platinum band indicator electrode and a saturated KCl/AgCl/Ag reference electrode. That redox combination probe was connected to a chemcadet pH meter (Cole-Parmer N-05983-00). Before connection, the mechanical and electrical zero points of the pH meter were adjusted in order to calibrate voltage scale. ORP readings were taken 15 min after introducing the electrode to the synthetic water or reactor sludge. This time was sufficient to achieve at least 96 % of electrode final response and was in the range of electrode stabilization time observed by Grune (20) during the measurement of digested sludge redox potentials. ORP values being dependent on temperature and pH, these parameters were recorded in parallel. Direct potential readings ($E_{AgCl/Ag}$) were converted to E_h by addition of the potential difference ($E_{reference}$) between AgCl/Ag reference electrode and the standard hydrogen electrode. That potential difference (in mV) was estimated from the formula $E_{reference} = 223.59 - 1.0062\ T$ where T is the temperature in C°. This equation was obtained from the standard potentials of silver-silver chloride electrodes tabulated by Bricker (21).

Enumeration of reactor sludge microflora.

Microbial counts of hydrogenophilic and acetoclastic methanogens, as well as obligate hydrogen producing acetogenic bacteria using propionate or butyrate, were realized by the most probable number (MPN) technique as described by Guyot et al. (22). The number of *p*-toluic acid users was determined using the same method, with *p*-toluic acid (0.25 $g\ l^{-1}$) as substrate.

Analytical methods.

COD, pH, temperature and VSS were determined according to APHA (23). All other procedures (sludge volume index, maximum settling velocity, granulometry, gas composition) have been described elsewhere (17, 19).

RESULTS AND DISCUSSION

Reactor start-up and performances

Digester feeding started with a wastewater containing 1 $g\ l^{-1}$ *p*-toluic acid (table 1, period 1). Because of its origin, the reactor sludge was assumed to be already adapted to *p*-toluic acid degradation. Nevertheless, it soon became apparent that there was practically no COD removal and the sludge was not capable of lowering the wastewater ORP efficiently, since reactor E_h remained positive (figure 1). On day 7, a small amount of glucose was added to the reactor influent to promote biological oxygen consumption (table 1, period 2). No improvement was observed, so on day 12 the wastewater was also supplemented with

$FeSO_4.7H_2O$ (table 1, period 3) but no COD removal occured until day 19 (almost 4 HRT cycles) and sludge E_h remained positive (figure 1).

The absence of reduction was surprising. It could arise from the use of an insufficient glucose and $FeSO_4$ loading rate (0.01 g glucose or $FeSO_4.7H_2O$.g $VSS^{-1}.d^{-1}$) or independently of the reducing agents added, from a biomass inhibition by *p*-toluic acid since benzenic aromatic compounds are recognized as potential toxicants for anaerobic digester sludges (17, 24, 25). In order to discriminate between toxicity and low amounts of reducing agents, on day 20, *p*-toluic acid concentration was decreased from 1 to 0.5 $g.l^{-1}$ while glucose and $FeSO_4$ concentrations were maintained at the same level (table 1, period 4).

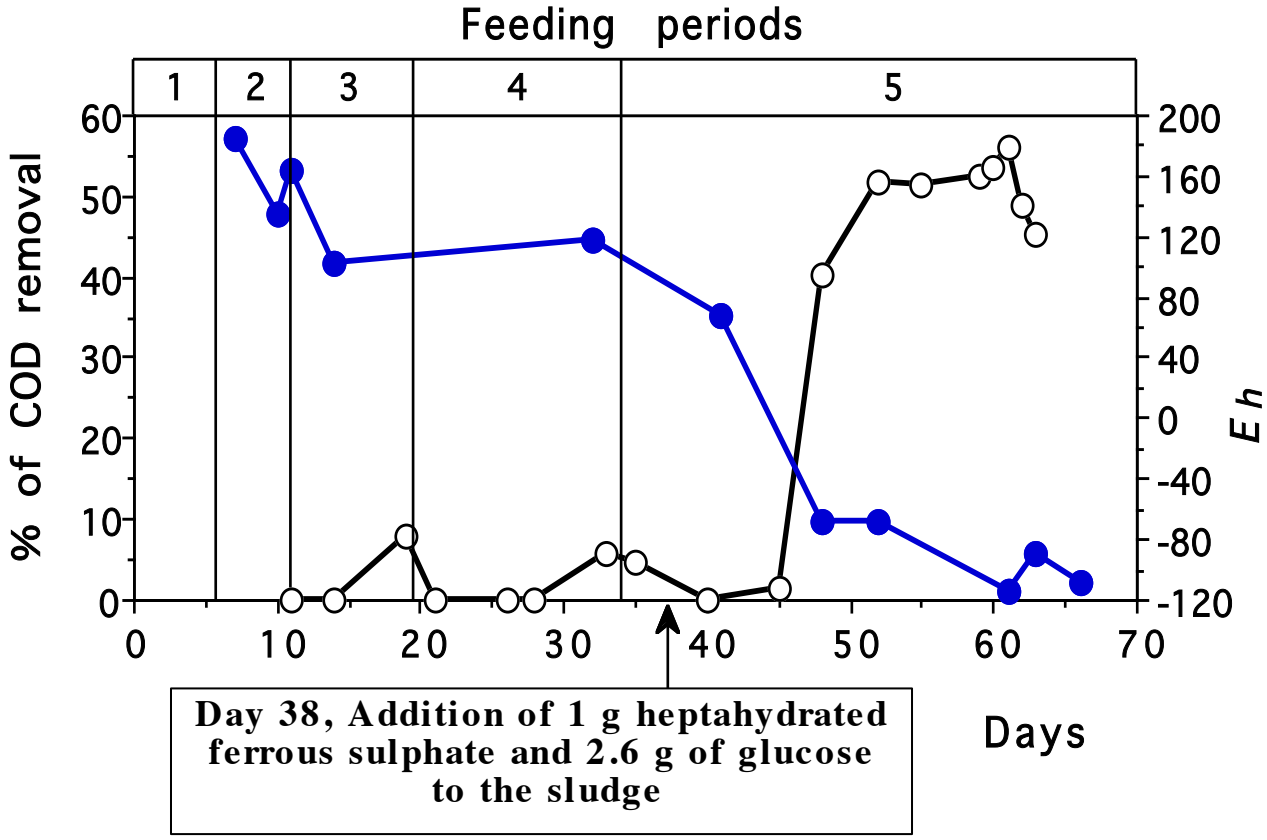


Fig 1. Evolution of COD removal (O) and sludge E_h (•) during reactor start-up

Table 2. Characterization of the different feeding and steady-state periods of the reactor (*).

Feeding periods	1, 2, 3	4	5	6
Influent COD $mg.l^{-1}$	2075 ± 308	994 ± 61	1050 ± 95	1014 ± 62
Influent pH	7.4 ± 0.4	7.0 ± 0.3	7.1 ± 0.3	7.0 ± 0.3
Influent E_h (mV)	**+250** ± 54	**+137**	**+171** ± 37	**+151** ± 44
Hydraulic Retention Time (days)	5.1 ± 1.0	5.8 ± 1.1	5.4 ± 1.3	5.2 ± 0.7
Organic Load (kg COD. kg $VSS^{-1}.d^{-1}$)	0.213	0.090	0.103	0.114
Sludge temperature (°C)	30.4 ± 2.6	34.7 ± 0.6	32.9 ± 1.8	32.2 ± 2.3
Sludge pH	7.4 ± 1.0	7.2 ± 0.07	7.3 ± 0.1	7.2 ± 0.1
Sludge E_h (mV)	**+161** ± 25	**+118**	**-95** ± 21	**-131** ± 50
COD removal (%)	**0 to 8**	**0 to 5.8**	**51.3** ± 3.4	**38.2** ± 7.0

(*) influent temperature is 6°C; values following ± are standard deviations;
E_h, redox potential relative to hydrogen reference.

During the following 17 days (3 HRT cycles), the reactor performances did not change and sludge E_h stayed positive (figure 1, table 2, period 4). Despite this result, *p*-toluic acid concentration was not decreased further because it was necessary to maintain an organic load compatible with anaerobic digestion.

The second alternative (low amounts of reducing agents) was then studied. Instead of increasing the glucose and $FeSO_4$ loading rates step by step by increasing their concentration in the wastewater, it was decided to add a large single dose of 1 g $FeSO_4.7H_2O$ (0.15 g $FeSO_4.7H_2O$.g VSS^{-1}) and 2.6 g glucose (0.38 g glucose.g VSS^{-1}) directly to the reactor sludge bed. Immediately after the addition, performed on day 38, sludge E_h decreased (figure 1, period 5). Fourteen days later, it attained a steady value of -95 mV (pH 7.3, 33°C). At the same time, COD removal started to increase, reaching finally at equilibrium (days 52 to 63) an average of 51.3% (figure 1, table 2, period 5). The decrease of E_h did not derive from a change in sludge temperature or pH since these parameters were fairly constant over the periods 4 to 5 (table 2). Following $FeSO_4$ addition, the color of the sludge bed turned from grey to black confirming the production of FeS. The electron donors (H_2, acetate, lactate, etc...) necessary for ferrous sulphate reduction by sulphate-reducers were probably generated by the anaerobic fermentation of glucose by other microorganisms. Part of the glucose was also probably used through aerobic respiration by some aerobic and facultative anaerobic bacteria.

For the rest of the study (days 98 to 269), glucose and $FeSO_4$ were no longer added to the reactor influent (table 1, period 6). In spite of the absence of these exogenous reducing agents, sludge E_h remained stable at the level achieved at the end of start-up (table 2, periods 5 and 6). This result proves that the redox buffering capacity generated by FeS production was still efficient 200 days after the addition of $FeSO_4$ to the reactor sludge bed. The decrease of the COD removal efficiency observed during the same period (table 2, period 6) was probably related to the lowering of iron availability. Indeed, after $FeSO_4$ elimination, iron concentration in the feed medium decreased from 20.4 to the 0.4 $mg.l^{-1}$ initially present in the tap water (see material and method).

Impact of $FeSO_4$ addition on granulation and characteristics of the reactor sludge.

The sludge used to inoculate the reactor mostly consisted of very small granules: 58 % had a diameter inferior to 150 μm while only 2 % had a diameter higher than 600 μm. After 236 days of operation and the direct addition of $FeSO_4$ to the reactor sludge bed, this distribution had not change much, since 61 % of the granules had a diameter inferior to 150 μm and 5 % higher than 600 μm. Nevertheless, during the same time, the settling characteristics of the sludge improved significantly as measured by the sludge volume index which decreased from 26.2 (day 0) to 16.3 $ml \cdot g^{-1}$ (day 236) and the maximum settling velocity which increased from 3.1 (day 0) to 4.84 $m \cdot h^{-1}$, (day 236). As a consequence, the formation of FeS resulting from the reduction of $FeSO_4$ did not appear to favour sludge granulation (increase of granule size), but to have a strong positive impact on sludge sedimentation probably due to an increase of the sludge specific gravity.

Sludge oxidation-reduction potential

The redox potential achieved by the sludge after the addition of $FeSO_4$ (E_h = -95 to -131 mV, pH 7.2 to 7.3, 32 to 33°C) remained high compared to the value of -330 mV, usually considered as the E_h upper limit above which methanogenic fermentation cannot occur. This value was determined by Smith and Hungate (1). It corresponds, according to them, to the E_h of samples of rumen fluid "when measured with a platinum electrode and calomel half cell". From this definition, it seems that the value obtained has been mis-called E_h and that it is most probably the direct potential reading of the calomel reference electrode ($E_{calomel}$) used. This assumption is confirmed by the absence of information about the conversion of the calomel direct readings to E_h and the fact that independent ORP measurements of several rumen contents (26) have given $E_{calomel}$ of the same order (-260 to -380 mV, pH 6.2 to 7.2). In these conditions, the real E_h of Smith and Hungate's rumen fluid would be -92.4 mV [= -330 + 237.6 where 237.6 is the potential difference between the saturated calomel electrode and the standard hydrogen electrode at 35°C given by Bricker (21)], which is comparable to the E_h observed in the reactor.

Dirasian *et al.* (27) and Blanc and Molof (28) have found, however, that the anaerobic digestion of activated sludge and the methanation of acetate and propionate proceeded satisfactorily only in a range of E_h from -212 to -313 mV (pH ≈ 7, 35°C) which is more negative than the range obtained during the present study (E_h were calculated from the reported $E_{calomel}$). In these works, there was no ambiguity about the reference electrodes used. Nevertheless, beside the nature of the reference electrode, another parameter of importance in ORP measurements is the time required to obtain an electrode stabilized response. The above mentioned authors have suggested that long adjustment periods (10 to 60 h) are required and so have performed their ORP determinations with electrodes permanently installed in the digesters. It is well known that prolonged contact of redox electrodes with sludges or solutions affects ORP measurements (29). For instance, sulphides which may have been present (even as traces) in the environments studied by the former authors, attack the surface of the platinum indicator electrodes leading to the measurement of artificially more negative ORPs (30). Thus, it appears that because of methodological inconsistencies, it is difficult to find relevant ORP determinations on anaerobic digestion in the literature.

The granular nature of the sludge in UASB reactors involves another constraint in the measurement of ORP. The values obtained correspond effectively to the ORP of the interstitial water around the granules but not to the ORP inside the granules which is probably more negative. This is supported by the microbial internal structure of the granules which creates a downward gradient of O_2 concentration and an upward gradient of pH from the periphery to the center (31, 32, 33). Both a decrease of O_2 concentration and an increase of pH influence directly redox potential by making it more negative (3, 34).

In any case, independently of the absolute value of the redox potential, counts of anaerobic bacteria performed on the reactor sludge long after the addition of $FeSO_4$ showed that the ORP level measured was compatible with the development of all members of the bacterial chain necessary for the degradation of *p*-toluic acid to CH_4 and CO_2 (table 3).

Table 3. MPN counts of the anaerobic bacteria present in the reactor sludge on day 236 of operation.

Metabolic group	MPN (bacteria.g VSS^{-1})
p-toluic acid degraders	$9.6\ 10^8$
Propionate degraders	$5.7\ 10^7$
Butyrate degraders	$6.7\ 10^7$
Hydrogenophilic methanogens	$6.7\ 10^8$
Acetoclastic methanogens	$2.5\ 10^8$

CONCLUSION

Ferrous sulphate has proved to be an excellent chemical to promote indirectly through FeS production a redox potential compatible with methanogenesis in the sludge of UASB reactors. In order to get an efficient reduction it should, preferentially, be added with an easily degradable organic compound as a source of electrons. Since the redox buffering capacity of the generated FeS lasts over a hundred days, a single addition of $FeSO_4$ could be made at the beginning of the reactor start-up period. A secondary positive effect of $FeSO_4$ addition is an improvement of the sludge settling properties.

Recently, in Mexico, $FeSO_4$ has been used successfully to solve a problem of redox potential which happened during the start-up of a 4800 m^3 UASB reactor treating brewery wastewater (Adalberto Noyola and Sara Mendoza, personal communication). After an abnormal discharge, the redox potential (as E_h) of the digester sludge increased to +44 mV (pH ≈ 7, 25.4°C) causing a deterioration of its acetoclastic activity. The dosage of 87 $mg.l^{-1}$ of $FeSO_4$ to the wastewater lowered sludge E_h to -100 mV (pH ≈ 7, 25.4°C) allowing the reactor to recover its previous performances.

Considering the economical point, if redox potential problems occur at full scale, it can be adviced to add ferrous sulphate as a mixture of powdered gypsum (35) or any other abundant natural sulphate ore and iron filings.

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